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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Dennis A. Carson et al.

Title: INDOLE COMPOUNDS USEFUL FOR THE TREATMENT OF CANCER

Attorney Docket No.: 103.022US1

PATENT APPLICATION TRANSMITTAL

BOX PATENT APPLICATION

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☒ **CONTINUATION-IN-PART (CIP)** of prior Patent Application No. 09/360,020 (under 37 CFR § 1.53(b))

Comprising:

☒ Specification (33 pgs, including claims numbered 1 through 48 and a 1 page Abstract).

☒ Formal Drawing(s) (15 sheets).

☒ Unsigned Combined Declaration and Power of Attorney (4 pgs).

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5 This application is a continuation-in-part of U.S. patent application Serial No. 09/360,020 filed July 23, 1999, and claims priority of U.S. provisional patent application Serial No. 60/189,976, filed March 16, 2000, both of which are incorporated by reference herein.

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the United States. In 1998, an estimated 185,000 men were diagnosed with prostate cancer, and more than 39,000 men died of the disease. See, S. H. Landis et al., Cancer Statistics, CA Cancer J. Clin., 48, 6 (1998). Although survival rates are good for prostate cancer that is diagnosed early, the treatments for advanced disease are limited to hormone ablation techniques and palliative care. Hormone ablation techniques (orchiectomy and anti-androgen treatments) generally allow only temporary remission of the disease. It usually recurs within 1-3 years of treatment, with the recurrent tumors no longer requiring androgens for growth and survival. D. G. Tang et al., Prostate, 32, 284 (1997). Therapy with conventional chemotherapeutic agents, such as progesterone, estramustine and vinblastine, has also not been demonstrated to be effective to halt progression of the disease.

The number of nonsteroidal anti-inflammatory drugs (NSAIDs) has increased to the point where they warrant separate classification. In addition to aspirin, the NSAIDs available in the U.S. include meclofenamate sodium, oxyphenbutazone, phenylbutazone, indomethacin, piroxicam, sulindac and tolmetin

for the treatment of arthritis; mefenamic acid and zomepirac for analgesia; and ibuprofen, fenoprofen and naproxen for both analgesia and arthritis. Ibuprofen, mefenamic acid and naproxen are used also for the management of dysmenorrhea.

The clinical usefulness of NSAIDs is restricted by a number of adverse effects. Phenylbutazone has been implicated in hepatic necrosis and granulomatous hepatitis; and sulindac, indomethacin, ibuprofen and naproxen with hepatitis and cholestatic hepatitis. Transient increases in serum aminotransferases, especially alanine aminotransferase, have been reported. All of these drugs, including aspirin, inhibit cyclooxygenase, that in turn inhibits synthesis of prostaglandins, which help regulate glomerular filtration and renal sodium and water excretion. Thus, the NSAIDs can cause fluid retention and decrease sodium excretion, followed by hyperkalemia, oliguria and anuria. Moreover, all of these drugs can cause peptic ulceration. See, Remington's Pharmaceutical Sciences, Mack Pub. Co., Easton, PA (18th ed., 1990) at pages 1115-1122.

There is a large amount of literature on the effect of NSAIDs on cancer, particularly colon cancer. For example, see H. A. Weiss et al., Scand J. Gastroent., 31, 137 (1996) (suppl. 220) and Shiff et al., Exp. Cell Res., 222, 179 (1996). More recently, B. Bellosillo et al., Blood, 92, 1406 (1998) reported that aspirin and salicylate reduced the viability of B-cell CLL cells *in vitro*, but that indomethacin, ketoralac and NS-398, did not.

C. P. Duffy et al., Eur. J. Cancer, 34, 1250 (1998), reported that the cytotoxicity of certain chemotherapeutic drugs was enhanced when they were combined with certain non-steroidal anti-inflammatory agents. The effects observed against human lung cancer cells and human leukemia cells were highly specific and not predictable; i.e., some combinations of NSAID and agent were effective and some were not. The only conclusion drawn was that the effect was not due to the cyclooxygenase inhibitory activity of the NSAID.

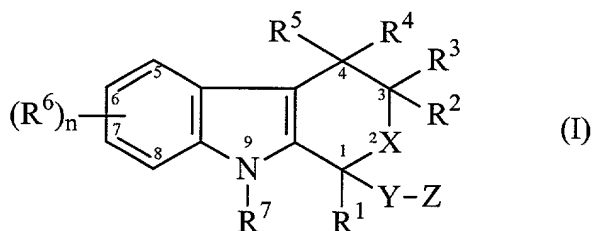
The Duffy group filed a PCT application (WO98/18490) on October 24, 1997, directed to a combination of a "substrate for MRP", which can be an anti-cancer drug, and a NSAID that increases the potency of the anti-cancer drug.

NSAIDs recited by the claims are acemetacin, indomethacin, sulindac, sulindac sulfide, sulindac sulfone, tolmetin and zomepirac. Naproxen and piroxicam were reported to be inactive.

Recently, W. J. Wechter et al., Cancer Res., 60, 2203 (2000) reported that the NSAID, R-flurbiprofen, inhibited progression of prostate cancer in the TRAMP mouse, a prostate cancer model. The Wechter group filed a PCT application (WO98/09603) on September 8, 1997, disclosing that prostate cancer can be treated with R-NSAIDs, including R(-)-etodolac and R-flurbiprofen. In contrast to R(-)-etodolac, the R-enantiomer of flurbiprofen and other (R)-2-aryl propionate NSAIDs are converted in the body to the anti-inflammatory S-enantiomers, and hence are pro-drugs of the NSAIDs, while R(-) etodolac is not per se an NSAID. Therefore, a continuing need exists for effective methods to employ these preliminary findings to develop new compounds to treat neoplastic disease, including prostate cancer and other cancers.

Summary of the Invention

The present invention provides indole compounds of formula (I):



wherein R^1 is lower alkyl, lower alkenyl, (hydroxy)lower alkyl, lower alkynyl, phenyl, benzyl or 2-thienyl, R^2 , R^3 , R^4 and R^5 are the same or different and are each hydrogen or lower alkyl; each R^6 is individually hydrogen, lower alkyl, hydroxy, (hydroxy)lower alkyl, lower alkoxy, benzyloxy, lower alkanoyloxy, nitro or halo, n is 1-3, R^7 is hydrogen, lower alkyl or lower alkenyl, X is oxy and thio, Y is carbonyl, $(CH_2)_{1-3}$, $(CH_2)_{1-3}C(O)$, or $(CH_2)_{1-3}SO_2$ and Z is $(\omega-(4\text{-pyridyl}))(C_2-C_4$ alkoxy), $(\omega-((R^8)(R^9) \text{ amino}))(C_2-C_4$ alkoxy), wherein R^8 and R^9 are each H, (C_1-C_3) alkyl or together with N are a 5- or 6-membered heterocyclic ring comprising 1-3

N(R⁸), S or nonperoxide O; an amino acid ester of (ω-(HO)(C₂-C₄))alkoxy, N(R⁸)CH(R⁸)CO₂H, 1'-D-glucuronyloxy; Y-Z is (CH₂)₁₋₃R⁸ wherein R⁸ is OH, (C₂-C₄)acyloxy, SO₃H, PO₄H₂, N(NO)(OH), SO₂NH₂, PO(OH)NH₂, or tetrazolyl; or a pharmaceutically acceptable salt thereof.

5 The present invention also provides a therapeutic method to inhibit the growth of cancer cells and/or to sensitize cancer cells to inhibition by a chemotherapeutic agent. The method comprises contacting cancer cells with an effective amount of the compound of formula (I), preferably in combination with a pharmaceutically acceptable carrier. The present compounds can be used to treat a
10 mammal afflicted with cancer, such as a human cancer patient, and are preferably administered in conjunction with a chemotherapeutic agent, such as an alkylating agent or an anti-androgen, radiation and/or other anti-cancer therapy.

 The present compounds are effective against hematopoietic cancers, such as leukemias and cancers of the bone marrow, including chronic lymphocytic leukemia
15 (CLL) and multiple myeloma (MM). The present compounds were unexpectedly found to be effective against cancer cells that express high levels of the nuclear hormone receptor, peroxisome proliferator activated receptor-γ, (PPAR-γ), and/or high levels of the anti-apoptotic proteins, Mcl-1 and/or Bag-1. Such cancer cells include at least some types of prostate cancer cells.

20 Activated PPAR-γ binds co-activator protein (CBP), a co-activator of the androgen receptor known to be overexpressed in hormone-resistant prostate cancer. Thus, compounds of formula (I) that activate PPAR-γ production can reduce the level of expression of the androgen receptor known to be over-expressed in hormone-resistant prostate cancer. Therefore, the present compounds can enhance
25 the efficacy of conventional anti-androgen therapy, and can act to inhibit the spread of prostate cancer.

 The present invention is based on the discovery by the inventors that racemic etodolac inhibits the viability of purified CLL or MM cells at concentrations that do not inhibit the viability of normal peripheral blood lymphocytes (PBLs). It was then
30 unexpectedly found that the R(-) enantiomer of etodolac is as toxic to CLL cells as

is the S(+) enantiomer. It was then found that etodolac synergistically interacted with fludarabine and 2-chloroadenosine to kill CLL cells at concentration at which the chemotherapeutic agents alone were inactive. Finally, it was found that both R(-)- and S(+)- etodolac inhibited a number of prostate cancer cell lines. Again the R(-)- enantiomer was at least as effective as the S(+)- “anti-inflammatory” enantiomer. This was unexpected since the R(-) enantiomer of etodolac does not possess significant anti-inflammatory activity and is not converted to the S(+) enantiomer to a significant extent *in vivo*. As noted above, the R-enantiomers of other R-2-arylpropionate NSAIDs are converted to the “active” anti-inflammatory S-enantiomers *in vivo*, and so function as pro-drugs for the NSAID.

The extent of inhibition was markedly related to the level of expression of PPAR- γ by the cell line. Cell lines with an elevated level of PPAR- γ expression were inhibited much more effectively than cell lines expressing relatively low levels of PPAR- γ , as disclosed in the working examples.

A compound of formula (I) is preferred for practice of the present therapeutic method that does not exhibit undesirable bioactivities due to inhibition of cyclooxygenase (COX) that are exhibited by some NSAIDs. However, the preferred compounds of formula (I) would not be considered NSAIDs by the art, as they would not exhibit significant anti-inflammatory activity.

Thus, the present invention also provides a method for determining whether or not a particular cancer patient, such as a prostate cancer patient, is amenable to treatment by a compound of formula (I), comprising isolating cancer cells and evaluating *in vitro* the relative level of PPAR- γ and/or Mcl-1 and/or Bag-1 relative to the level in a cancer cell line, such as prostate cancer cell line, known to be susceptible to treatment by a compound of formula (I).

The present invention also provides a method to determine the ability of a test agent to inhibit cancer cells, such as prostate cancer cells, comprising contacting a population of cancer cells, as from a prostate cancer cell line, with said agent and determining whether the agent increases expression of PPAR- γ , or decreases the expression of Mcl-1 and/or Bag-1 (or does both). The present invention also

provides a general multilevel screening method to evaluate etodolac analogs, other NSAIDs or other agents for their ability to inhibit cancer, preferably etodolac-sensitive cancers, such as prostate cancer, CLL and MM. Agents that exhibit a positive activity, preferably at least equal to that of R(-)-etodolac, or do not exhibit a negative activity, e.g., are no more active than R(-)-etodolac, are passed to the next screen.

Test agents are first evaluated for their ability to competitively inhibit the binding of etodolac, e.g., radiolabeled R(-) etodolac to its receptor(s) on etodolac-sensitive cancer cells such as CLL cells. Agents that can compete effectively with R(-) etodolac for etodolac binding site(s) on the cells are then evaluated in an assay to determine if they can increase Ca^{+2} uptake in cancer cells, such as CLL cells, preferably as effectively as R(-) etodolac. Agents that can induce intracellular Ca^{+2} uptake are screened to determine if they can induce chemokinetic activity (chemokinesis or chemotaxis) in a population of lymphocytes, such as B-CLL lymphocytes, preferably as effectively as R(-) etodolac. Agents that are positive in this screen are then evaluated to determine if they can induce apoptosis or pro-apoptotic factors, such as increased caspase activity in cancer cells, such as CLL cells and other cancer cells known to be etodolac sensitive, at least as effectively as R(-) etodolac.

Agents that test positive in this screen are evaluated for their ability to deplete lymphocytes in mice, and those that are no more active than R(-) etodolac are then evaluated in animal models of cancer to see if they can inhibit the induction of, or spread of cancer.

As used herein with respect to cancer or cancer cells, the term "inhibition" or "inhibit" includes both the reduction in cellular proliferation, blockage of cellular proliferation, or killing some or all of said cells. Thus, the term can be used in both the context of a prophylactic treatment to prevent development of cancer or as a treatment that will block, or slow the spread of established cancer. Whether or not the level of expression of a marker of susceptibility to etodolac treatment is sufficiently elevated to continue treatment with etodolac or an analog thereof is

determined by comparison between the relative levels of expression of said marker in resistant and susceptible cancer cell lines, as disclosed hereinbelow.

Brief Description of the Figures

5 Figure 1 is a graph depicting the sensitivity of normal peripheral blood lymphocytes (PBL) to racemic etodolac.

 Figure 2 is a graph depicting the sensitivity of CLL cells to racemic etodolac.

 Figure 3 is a graph depicting the synergistic effect of a combination of
10 racemic etodolac and fludarabine against CLL cells.

 Figure 4 is a graph depicting the synergistic effect of a combination of 50 μ M etodolac with 10 μ M 2CdA or 10 mM Fludara against CLL cells.

 Figure 5 is a graph depicting the sensitivity of CLL cells to S- and R-etodolac.

15 Figures 6 and 7 depict the viability of CLL cells from two patients before and after etodolac administration.

 Figure 8 depicts a flow cytometric analysis of CLL cells before and after etodolac treatment.

 Figures 9 and 10 depict the selective action of R(–)-etodolac against MM
20 cells from two patients.

 Figure 11 is a photocopy of a SDS-PAGE gels demonstrating that etodolac induces a rapid downregulation in Mcl-1 (Panel A) and Bag-1 (Panel B), that is blocked by MG-132.

 Figure 12 is a photocopy of an SDS-PAGE gel depicting expression of
25 PPAR- γ by seven cancer cell lines.

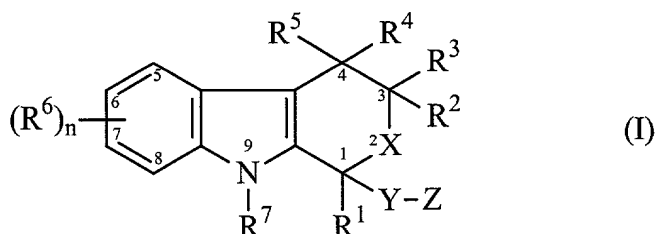
 Figure 13 is a graph depicting induction of PPAR- γ expression by etodolac and indomethacin.

 Figure 14 is a graph depicting expression of CD36 induced by etodolac and TGZ, in the presence and absence of TPA in human monocytes.

Figure 15 is a photocopy of sections of prostate cancer tissue, untreated (A) or treated (B, C, D) with etodolac.

Detailed Description of the Invention

- 5 Indole compounds of the present inventions include compounds of formula (I):



- wherein R¹ is selected from the group consisting of lower alkyl, lower alkenyl, (hydroxy)lower alkyl, lower alkynyl, phenyl, benzyl and 2-thienyl, R², R³, R⁴ and R⁵ are the same or different and are each selected from the group consisting of hydrogen and lower alkyl, each R⁶ is individually selected from the group consisting of hydrogen, lower alkyl, hydroxy, (hydroxy)lower alkyl, lower alkoxy, benzyloxy, lower alkanoyloxy, nitro and halo, n is 1-3, R⁷ is selected from the group consisting of hydrogen, lower alkyl and lower alkenyl, X is selected from the group consisting of oxy and thio, Y is selected from the group consisting of carbonyl (CH₂)₁₋₃, (CH₂)₁₋₃SO₂ or (CH₂)₁₋₃C(O), and Z is selected from the group consisting of hydroxy, lower alkoxy optionally substituted with OH, 4-pyridyl, amino, lower alkylamino, di(lower alkyl)amino, or N-morpholino; amino, lower alkylamino, [(carboxy)(lower alkyl)]amino, di(lower)alkylamino and phenylamino, or Y-Z is (CH₂)₁₋₃R⁸ wherein R⁸ is OH, (C₂-C₄)acyloxy, SO₃H, PO₄H₂, N(NO)(OH), SO₂NH₂, PO(OH)NH₂, or tetrazolyl or a pharmaceutically acceptable salt thereof. Lower alkyl, alkenyl, alkanoyl, etc. indicates a branched, cyclic or straight chain C₁-C₆ group, preferably a C₁-C₄ group, including cycloalkyl and (cycloalkyl)alkyl. (Hydroxy)lower alkyl or alkoxy is preferably 1- or 2- hydroxyethyl.

- As discussed above, the relatively low water solubility of the R(-) enantiomer of etodolac can reduce its usefulness against cancer when administered

orally, or in an aqueous vehicle. Therefore, the present invention also provides novel indole compounds that exhibit enhanced water solubility and/or bioavailability over the free acid or the simple alkyl esters of etodolac. Such analogs include (pyridinyl) lower alkyl esters, (amino)lower alkyl esters, (hydroxy)lower alkyl esters and 1'-D-glucuronate esters of etodolac, e.g., compounds of formula (II) wherein (a) Y is carbonyl and (b) Z is (ω -(4-pyridyl)(C₂-C₄ alkoxy), (ω -((R⁸)(R⁹) amino)(C₂-C₄ alkoxy), wherein R⁸ and R⁹ are each H, (C₁-C₃)alkyl or together with N are a 5- or 6-membered heterocyclic ring comprising 1-3 N(R⁸), S or nonperoxide O; an amino acid ester of (ω -(HO)(C₂-C₄)alkoxy, e.g., the L-valine or L-glycine ester of 2-hydroxyethoxy, 1'-D-glucuronyloxy; and the pharmaceutically acceptable salts thereof, e.g., with organic or inorganic acids. Other analogs of increased water solubility include amino acid amides, where Y is carbonyl and Z is N(R⁸)CH(R⁸)CO₂H, and the pharmaceutically acceptable salts thereof.

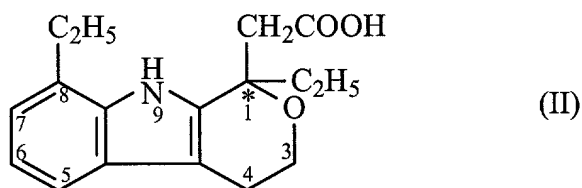
Such compounds can be prepared as disclosed in U.S. Pat. No. 3,843,681, U.S. Pat. Application Serial No. 09/313,048, Ger. Pat. No. 2,226,340 (Amer. Home Products), R. R. Martel et al., Can. J. Pharmacol., **54**, 245 (1976); Demerson et al., J. Med. Chem., **19**, 391 (1976); PCT application Serial No. US/00/13410 and Rubin (U.S. Pat. No. 4,337,760).

The resolution of racemic compounds of formula (I) can be accomplished using conventional means, such as the formation of a diastereomeric salt with a optically active resolving amine; see, for example, "Stereochemistry of Carbon Compounds," by E. L. Eliel (McGraw Hill, 1962); C. H. Lochmuller et al., J. Chromatog., **113**, 283 (1975); "Enantiomers, Racemates and Resolutions," by J. Jacques, A. Collet, and S. H. Wilen, (Wiley-Interscience, New York, 1981); and S. H. Wilen, A. Collet, and J. Jacques, Tetrahedron, **33**, 2725 (1977). For example, the racemate has been resolved by fractional crystallization of RS-etodolac using optically active 1-phenylethylamine and HPLC has been used to determine racemic etodolac and enantiomeric ratios of etodolac and two hydroxylated metabolites in urine (U. Becker-Scharfenkamp et al., J. Chromatog., **621**, 199 (1993)). B. M.

Adger et al. (U.S. Patent No. 5,811,558), disclosed the resolution of etodolac using glutamine and N(C₁-C₄ alkyl)-glutamine salts.

Etodolac itself (1,8-diethyl-1,3,4,9-tetrahydro[3,4-6]indole-1-acetic acid) is a NSAID of the pyranocarboxylic acid class, that was developed in the early 1970s.

- 5 Its structure is depicted as formula (II), below, wherein (*) denotes the chiral center. See also, The Merck Index, (11th ed.), at page 608.



- The pharmacokinetics of etodolac have been extensively reviewed by D. R. Brocks et al., Clin. Pharmacokinet., 26, 259 (1994). Etodolac is marketed as the racemate. The absolute configurations of the enantiomers were found to be S-(+) and R(-), which is similar to that for most other NSAIDs. However, Demerson et al., J. Med. Chem., 26, 1778 (1983) found that the S(+)-enantiomer of etodolac possessed almost all of the anti-inflammatory activity of the racemate, as measured by reduction in paw volume of rats with adjuvant polyarthritis, and prostaglandin synthetase inhibitory activity of the drug. No anti-inflammatory activity was discernible with the (-)-enantiomer, and it is not converted significantly to the S(+) enantiomer *in vivo*. Hence, R(-) etodolac is not a NSAID. However, as disclosed below, R(-) etodolac paradoxically was found to have potent activity against cancer cells that is at least equivalent to that of the S(+) enantiomer.

- 20 Etodolac possesses several unique disposition features due to their stereoselective pharmacokinetics. In plasma, after the administration of RS-etodolac, the concentrations of the “inactive” R-enantiomer of etodolac are about 10-fold higher than those of the active S-enantiomer, an observation that is novel among the chiral NSAIDs. See, D. R. Brocks et al., Clin. Pharmacokinet., 26, 259
25 (1994). After a 200 mg dose in six elderly patients, the maximum plasma concentration of the R-enantiomer was about 33 μM. In contrast, the maximum

concentration of the S-enantiomer was 5-fold lower. The typical dosage of the racemic mixture of etodolac is 400 mg BID, and the drug has an elimination half-life between 6-8 hours. Moreover, it is likely that the administration of the purified R-enantiomer will not display the side effects associated with cyclooxygenase (COX) inhibitors, such as ulcers and renal insufficiency, and thus can be given at considerably higher dosages. Nonetheless, the relatively low solubility of R(-)-etodolac in water can impede attaining plasma levels in humans that can inhibit cancer cells, particularly prostate cancer cells. However, the compounds of formula (I) can be dissolved in water and other aqueous carriers at substantially higher concentrations than R(-) etodolac.

The compounds of formula (I) can also be prepared in the form of their pharmaceutically acceptable salts or their non-pharmaceutically acceptable salts. The non-pharmaceutically acceptable salts are useful as intermediates for the preparation of pharmaceutically acceptable salts. Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects. Examples of such salts are (a) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; and salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (b) salts formed from elemental anions such as chlorine, bromine, and iodine. Preferred carboxylic acid salts are those of hydrophilic amines, such as glucamine or N-(C₁-C₄)alkylglucamine (see, Adger et al. (U.S. Pat. No. 5,811,558)).

The magnitude of a prophylactic or therapeutic dose of a compound or compounds of formula (I) in the acute or chronic management of cancer, i.e., prostate cancer, will vary with the type and/or stage of the cancer, the adjunct chemotherapeutic agent(s) or other anti-cancer therapy used, and the route of

administration. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, condition, and response of the individual patient. In general, the total daily dose range for a compound or compounds of formula (I), for the conditions described herein, is from about 50 mg to about 5000 mg, in single or
5 divided doses. Preferably, a daily dose range should be about 100 mg to about 4000 mg, most preferably about 1000-3000 mg, in single or divided doses, e.g., 750 mg every 6 hr of orally administered compound. This can achieve plasma levels of about 500-750 μ M, which can be effective to kill cancer cells. In managing the patient, the therapy should be initiated at a lower dose and increased
10 depending on the patient's global response. It is further recommended that infants, children, patients over 65 years, and those with impaired renal or hepatic function initially receive lower doses, particularly of analogs which retain COX inhibitory activity, and that they be titrated based on global response and blood level. It may be necessary to use dosages outside these ranges in some cases. Further, it is noted
15 that the clinician or treating physician will know how and when to interrupt, adjust or terminate therapy in conjunction with individual patient response. The terms "an effective inhibitory or amount" or "an effective sensitizing amount" are encompassed by the above-described dosage amounts and dose frequency schedule.

Any suitable route of administration may be employed for providing the
20 patient with an effective dosage of a compound of formula (I). For example, oral, rectal, parenteral (subcutaneous, intravenous, intramuscular), intrathecal, transdermal, and like forms of administration may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, patches, and the like. The compound may be administered prior to, concurrently with, or after
25 administration of chemotherapy, or continuously, i.e., in daily doses, during all or part of, a chemotherapy regimen. The compound, in some cases, may be combined with the same carrier or vehicle used to deliver the anti-cancer chemotherapeutic agent.

Thus, the present compounds may be systemically administered, e.g., orally,
30 in combination with a pharmaceutically acceptable vehicle such as an inert diluent

or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more excipients and used in the form of ingestible
5 tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically
10 useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrated agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening
15 agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the
20 solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. Tablets, capsules, pills, granules, microparticles and the like can also comprise an enteric coating, such as a coating of one of the Eudragit® polymers, that will permit release of the active compound(s) in the intestines, not in the acidic environment of the stomach. This can be
25 advantageous in the case of elderly or frail cancer patients treated with any compound that retains a significant COX-inhibitory activity, and concomitant ulceration.

A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring
30 such as cherry or orange flavor. Of course, any material used in preparing any unit

dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a non-toxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, non-toxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In

the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

5 Useful dosages of the compounds of formula I can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Patent No. 4,938,949.

10 Due to the ability of compounds of formula (I) that elevate PPAR- γ levels, to lower the expression of the androgen receptor known to be overexpressed in hormone-refractory prostate cancer, compounds that upregulate PPAR- γ are advantageously used in combination with steroidal and non-steroidal anti-androgens used in the treatment of prostate cancer. These compounds include leuprolide or goserelin acetate, bicalutamide and flutamide, nilutamide, cycloproterone acetate,
15 among others.

 Due to the ability of compounds of formula (I) that reduce PPAR- γ levels to sensitize prostate cancer cells to killing by conventional chemotherapeutic agents, such compounds can be employed with chemotherapeutic agents used to treat cancers such as prostate cancer, including estramustine, vinblastine, mitoxanthrone,
20 prednisone and the like, or melphalan to treat MM. Other chemotherapeutic agents, irradiation or other anti-cancer agents such as anti-tumor antibodies, or cytokines can be used with the present compounds. See, e.g., Remington's Pharmaceutical Sciences (18th ed. 1990) at pages 1138-1162.

 The invention will be further described by reference to the following detailed
25 examples.

**Example 1. Sensitivity of Normal Peripheral Blood Lymphocytes
and CLL Cells to Etodolac**

30 Mononuclear cells were isolated from the peripheral blood of B-CLL patients and normal donors using density gradient centrifugation (Ficoll-Paque).

Cells were cultured at 2×10^6 cells per mL in RPMI with 20% autologous plasma in 96-well plates with or without the indicated μM concentrations of etodolac (racemic, S-etodolac, R-etodolac) and in combination with 2-chloro-2'-deoxyadenosine (2CdA) or fludarabine. At indicated times (12, 24, 36, 48, 60, 72 hours), viability assays were performed using the erythrocin B exclusion assay, as described by D. Carson et al., PNAS USA, 89, 2970 (1992).

As shown in Figure 1, significant death of normal PBLs occurred only at 800 μM racemic etodolac, a concentration which cannot be obtained *in vivo*.

Peripheral blood lymphocytes from a normal donor were cultured with 1.0 mM etodolac for 24 hours. Then B lymphocytes were identified by staining with anti-CD19 antibody, and viability was assessed by DiOC₆ fluorescence. Etodolac under these conditions did not reduce the viability of the normal B cells, compared to control cultures. When the same viability assay was run with purified CLL cells from the peripheral blood of a CLL patient, the results were different. As shown in Figure 2, 50% of the CLL cells were killed by a 48 hour exposure to 200 μM racemic etodolac. More than 95% of the treated cells were malignant B lymphocytes.

Example 2. Synergistic Combinations of Etodolac and Chemotherapeutic Agents

Fludarabine is a nucleoside analog commonly used for the treatment of CLL. In this experiment the *in vitro* survival of CLL cells at the indicated time points was compared in cultures containing medium alone ("Con", squares), fludarabine 10 nM (diamonds), etodolac 10 μM (closed circles), and fludarabine 10 nM plus etodolac 10 μM (open circles). The two drugs together exhibited a synergistic cytotoxic effect. Figure 3 shows that the combination killed 50% of CLL cells during 48 hours of culture, while either drug alone was ineffective. Figure 4 demonstrates synergy between 50 μM etodolac and 10 nM 2-chlorodeoxyadenosine and fludarabine, under the same test conditions.

Example 3. Effect of R(-) and S(+) Etodolac Against CLL Cells

- Etodolac tablets were ground in a mortar and extracted from the formulation using ethyl acetate. The resulting racemic mixture of enantiomers was separated into R and S isomers on a preparative scale by fractional crystallization by the procedure of Becker-Scharfenkamp and Blaschke, *J. Chromatog.*, **621**, 199 (1993). Thus, the racemic mixture solid was dissolved in absolute 2-propanol and S-1-phenylethylamine was added to the solution. The resulting salt solution was stored in the refrigerator for 4 days. The crystalline white salt product was filtered and washed with cold 2-propanol and recrystallized two more times from 2-propanol.
- The same procedure was repeated for the R isomer only using R-1-phenylethylamine as the resolving agent. Finally, the R and S salts were decomposed using 10% sulfuric acid (v/v) and extracted with ethyl acetate. The chiral purity of each isomer was verified by HPLC using a Chiral-AGP column from ChromTech.
- The toxicities of the two enantiomers to CLL cells cultured in RPMI 1640 medium with 10% autologous plasma were compared at the indicated concentrations and time points, as shown in Figure 5. The R- and S-enantiomers are equivalently cytotoxic to the CLL cells.

Example 4. Viability of CLL Cells Before and After Etodolac Treatment

- Heparinized blood was taken from two patients (JK and NA) with CLL. Then each patient immediately took a 400 mg etodolac tablet, and a second tablet 12 hours later. After another 12 hours, a second blood specimen was obtained. The CLL cells were isolated and their survival *in vitro* were compared in RPMI 1640 medium containing 10% autologous plasma, as described in Example 1. The circles show CLL cells before etodolac treatment. In Figures 6-7, the upward pointing triangles represent CLL cell viability after etodolac treatment, wherein the cells are dispersed in medium containing the pretreatment plasma. The downward pointing triangles are CLL cells after treatment maintained in medium with the post-treatment plasma.

Figure 6 shows the different survivals of the two cell populations from patient JK. Note that the cells after treatment had a shortened survival compared to the cells before treatment. Figure 7 shows a less dramatic but similar effect with patient NA. Figure 8 is a flow cytometric analysis of CLL cells from patient JK before and after etodolac treatment. DiOC₆ is a dye that is captured by mitochondria. When cells die by apoptosis, the intensity of staining decreases. The X axis on the four panels in Figure 8 shows the DiOC₆ staining. An increased number of dots in the left lower box indicates cell death by apoptosis. If one compares the cells taken from the patient before etodolac treatment, and after etodolac treatment, one can see that the number of dots in the left lower box is much higher after the drug. This effect is detectable at 12 hours, and increases further after 24 hours.

To conduct the flow cytometric analysis, the mitochondrial transmembrane potential was analyzed by 3,3' dihexyloxacarbonycyanide iodide (DiOC₆), cell membrane permeability by propidium iodide (PI)³ and mitochondrial respiration by dihydrorhodamine 123 (DHR) (See J. A. Royall et al., Arch. Biochem. Biophys., 302, 348 (1993)). After CLL cells were cultured for 12 or 24 hours with the indicated amount of etodolac, the cells were incubated for 10 minutes at 37°C in culture medium containing 40 nM of DiOC₆ and 5 µg/ml PI. Cells were also cultured for 3 hours with the indicated amount of etodolac, spun down at 200 × g for 10 minutes and resuspended in fresh respiration buffer (250 mM sucrose, 1 g/L bovine serum albumin, 10 mM MgCl₂, 10 mM K/Hepes, 5 mM KH₂PO₄ (pH 7.4)) and cultured for 10 minutes at 37° C with 0.04% digitonin. Then cells were loaded for 5 minutes with 0.1 µM dihydrorhodamine (DHR). Cells were analyzed within 30 minutes in a Becton Dickinson FAC-Scalibur cytofluorometer. After suitable comprehension, fluorescence was recorded at different wavelength: DiOC₆ and DHR at 525 nm (FL-1) and PI at 600 nm (FL-3).

As a general matter a reduction of 10% in the survival of the post-treatment malignant cells, compared to the pretreatment malignant cells, at 16 hours after

culture *in vitro* is considered a “positive” in this test, and indicates the use of etodolac, i.e., R(-) etodolac in CLL or other cancer therapy.

Example 5. Ability of R(-)-Etodolac to Selectively Kill MM Cells

- 5 Bone marrow was obtained from two patients with multiple myeloma. The marrow contained a mixture of malignant cells, as enumerated by high level expression of the CD38 membrane antigen, and normal cells. The suspended marrow cells were incubated for 72 hours in RPMI 1640 medium with 10% fetal bovine serum, and various concentrations of the purified R-enantiomer of etodolac.
- 10 Then the dead cells were stained with propidium iodide, and the multiple myeloma cells were stained with fluorescent monoclonal anti-CD38 antibodies. The data were analyzed by fluorescent activated cell sorting. Figures 9-10 show that R-etodolac did not kill the normal bone marrow cells (light bars), but dose-dependently killed the multiple myeloma cells (dark shaded areas), in the marrow
- 15 cells from both patients.

Example 6. Etodolac Cytotoxicity to Cancer Cell Lines

- Table 1 summarizes the cytotoxic effects of R(-)-etodolac toward prostate cancer cell lines and one colon cancer cell line are indeed within clinically
- 20 achievable concentrations, given that a 1 gram dosage of R(-)-etodolac should yield a maximal plasma concentration in a human subject of about 400 μ M. The fact that the R(-)- and S(+)-enantiomers are both cytotoxic indicates that the anti-prostate cancer activity is COX independent. Note that R(-)-etodolac, which is devoid of anti-inflammatory activity, nonetheless is more toxic to prostate cancer cells than is
- 25 S(+) etodolac.

Table 1

Cell line	Origin	Etodolac R/S	Etodolac R	Etodolac S	Phenoty
PC-3	Prostate	340 ± 20*	150 ± 15*	800 + 30*	Sensitive
5 LNCaP-FGC	Prostate	400 ± 35	270 ± 50	220 ± 20	Sensitive
Alva-31	Prostate	>1000	>1000	>1000	Resistant
OVCAR-3	Ovarian	>1000	>1000	>1000	Resistant
MDA-MB-231	Breast	>1000	>1000	>1000	Resistant
HCT-116	Colon	450 ± 15	280 ± 20	420 ± 50	Sensitive
10 SW260	Colon	1000 ± 120	ND	ND	Resistant
A549	Lung	>1000	>1000	>1000	Resistant

* IC₅₀ (μM) of Etodolac R/S, R or S. Cytotoxicity was assessed by MTT assay after three days continuous exposure to decreasing concentrations of the agent. The results were confirmed by FACS using propidium iodide uptake.

Example 7. Etodolac Downregulation of Mcl-1 and Bag-1

As planar hydrophobic compounds, etodolac and other NSAIDS can readily insert into cell and organ membranes, and can disrupt their structure and function (S. B. Abramson et al., *Arthritis and Rheumatism*, **32**, 1 (1989)). The proteins Mcl-1 and Bag-1 are anti-apoptotic members of the bcl-2 family that are found in mitochondria (X. Wang et al., *Exp. Cell Res.*, **235**, 210 (1997)). As early as two hours after incubation with 100 μM etodolac, Mcl-1 and Bag-1 levels fell in an etodolac sensitive prostate cancer cell line (LNCaP). The fall in Mcl-1 and Bag-1 levels was prevented by co-incubation of the prostate cells with 5.0 μM MG-132, a recently described inhibitor of the proteasome (Figure 11, Panels A and B, respectively) (D. H. Lee et al., *Trends Cell Biol.*, **8**, 397 (1998)). Detergent lysates (20 μg per lane) were subjected to SDS-PAGE and immunoblotted with anti-Mcl-1 and anti-Bag-1 antibodies. Pre-incubation of the cells with Z-VAD, a broad-spectrum caspase inhibitor, did not prevent the Mcl-1 and Bag-1 downregulation. Etodolac incubation did not alter Bcl-2 and Bax levels (data not shown). Thus, etodolac did not interfere with Mcl-1 synthesis, but probably accelerated its

turnover. Both R- and S-etodolac induced Mcl-1 degradation at equivalent concentrations.

Example 8. Expression of PPAR- γ in Cancer Cell Lines

5 Although etodolac has not been previously studied, high concentrations of other NSAIDs have been reported to activate the nuclear hormone receptor PPAR- γ (J. M. Lehmann et al., J. Biol. Chem., **272**, 3406 (1997). Moreover, maximal activation of PPAR- γ induces apoptosis in human macrophages (G. Chinetti et al., J. Biol. Chem., **273**, 25579 (1998). Therefore, it was of interest to determine if

10 prostate cells express PPAR- γ , and to compare the expression level with other cancer types. Detergent lysates (20 μ g per lane) obtained from subconfluent cell lines were subjected to SDS-PAGE and immunoblotted with anti-PPAR- γ antibodies. To normalize the PPAR- γ content, the membrane was reblotted with an anti-actin monoclonal antibody. Lane 1: PC-3, Lane 2: SW260, Lane 3: A549,

15 Lane 4: MDA-MB-231, Lane 5: Alva-31, Lane 6: LNCaP, Lane 7: HCT-116 (see Table 1). It was observed that some etodolac-susceptible prostate cells (PC3 especially) expressed remarkably high levels of immunoreactive PPAR- γ (Figure 12).

Example 9. Activation of PPAR- γ by Etodolac

20 RAW264.7 cells were transfected at a density of 3×10^5 cells/ml in six well plates using lipofectamine with the PPAR- γ expression vector pCMX-PPAR- γ (0.1 μ g), and the PPAR- γ reporter construct (AOx)₃-TK-Luc (1 μ g) as previously described by M. Ricote et al., Nature, **391**, 79 (1998). Cells were treated for 24

25 hours with the compounds indicated on Figure 13, harvested and assayed for luciferase activity. Results are expressed as the mean \pm SD. As shown in Figure 13, both the R- and S- enantiomers of etodolac activated a PPAR- γ reporter gene construct at concentrations readily achieved in human plasma after *in vivo* administration. THP-1 human monocytic cells (ATCC) were incubated in the

30 presence or absence of phorbol ester (40 ng TPA) and 200 μ M racemic etodolac or

20 μ M troglitazone. After three days of culture, the surface expression of the scavenger receptor CD36 was measured by flow cytometry. As shown in Figure 14, both R- and S-etodolac caused the expression of CD36, a marker of PPAR- γ activation, in the human cell line THP-1 during macrophage differentiation.

5

Example 10. Etodolac Treatment of Prostate Cancer Tissue Samples

Freshly obtained prostatectomy samples were cut into 3 mm³ pieces, and incubated for 72 hours in RPMI-1640 supplemented with 10% FBS and antibiotics in the absence (A, 400X) or presence of racemic etodolac (B, 400X) or the purified R enantiomer (C, 400X; and D, 630X). The tissues were next fixed in 4% paraformaldehyde in PBS, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Figure 15A shows the infiltrating tumor cells (large nuclei) and some residual normal epithelium. Figures 15 B to 15 D show the effect of etodolac: note the abundant presence of pyknotic apoptotic nuclei (dark arrows, B and D), and the disintegration of the neoplastic glandular architecture (B+C). Etodolac was found to be selectively toxic to the tumor cells, but did not affect normal basal cells. The racemic mixture (R/S) and the purified R and S analogs were found both active.

Example 11. Prospective Protocol for Screening to Identify Etodolac Analogs

A. Screening of analogs by competition against radiolabeled R-etodolac

Etodolac-sensitive chronic lymphocytic leukemia [CLL] cells, or other cancer cells, will be utilized for drug screening in radioreceptor binding assay. In brief, frozen CLL cells will be washed three times in Hanks' Balanced Salt Solution (HBSS) and resuspended in HBSS-HEPES. The assay will be done in a total volume of 200 μ l containing approximately 2 million cells, [3H]-R-Etodolac [sp.act.20-25 Ci/mmol, prepared by Sibtech] and potential competitors or buffer are incubated in at varying temperatures [4 and 37°C] and times [0-60 minutes]. For each sample, triplicate 50 μ l aliquots will be layered over 300 μ l 20% sucrose in HBSS-HEPES in 1.5 ml polypropylene snap top tubes and pelleted for 2.5 minutes

at 15000 rpm in a Beckman microfuge. This procedure rapidly separates the cell-bound and cell-free etodolac. The tube tips will be cut off and the cell pellets will be solubilized and counted in a scintillation counter. Some of the incubation mixtures will contain excess unlabeled etodolac as a control. Specific binding is the difference in the bound cpm in tubes containing the radiolabeled etodolac minus the cpm in the tubes containing the radiolabeled etodolac and the excess cold competitor etodolac. Test agents are compared to the unlabeled cold competitor etodolac for their abilities to inhibit radiolabeled etodolac binding. Compounds that can inhibit the binding of radiolabeled etodolac to its receptor(s) are advanced to the next screen.

B. Intracellular Ca^{2+} mobilization in CLL

Increase of intracellular calcium levels in CLL cells by test compounds such as etodolac analogs will be measured by a flow cytometric assay (FACS) and by using a fluorometric imaging plate reader system (FLIPR, Molecular Devices Corp., Sunnyvale, CA) using the Fluo-4 dye (Molecular Probes). Briefly, CLL cells ($5 \times 10^6/\text{ml}$) will be loaded for 30 min with 4 μM of Fluo-4 at 37°C in serum-free medium, washed twice, and resuspended for an additional 30 min in normal cell culture medium. The loaded cells will be then mixed in FACS tubes with medium containing a test agent, and immediately thereafter the fluorescence will be followed by FACS analysis over a period of 3 minutes. For high-throughput screening (HTS) assays, the FLIPR-based assay will allow screening in a 96-well plate format, using the same fluorometric dye (Fluo-4). Positive controls will be performed using the calcium ionophore ionomycin at 50 ng/ml final concentration, with chemokines such as SDF-1 and IP-10, and with anti-IgM cross-linking antibodies. Compounds that increase the Ca^{+2} uptake by CLL cells, preferably to at least the level induced by R(-)-etodolac are advanced to the next screen.

C. Chemotaxis and chemokinesis assays

Cell migration will be measured in a 24-well modified Boyden chamber (Transwell, Corning-Costar, NY). The recombinant human IP-10 chemokine (R&D Systems, McKinley Place, NE) will be diluted in RPMI-1640 medium at 200 ng/ml,

and used to evaluate the chemotactic properties of lymphocytes from B-CLL patients. Polycarbonate membranes with pore size of 3 mm will be used. A total of 600 mL of chemokines or control medium will be added to the bottom wells, and 100 mL of 2 to 5.0×10^6 cells/ml cells resuspended in RPMI-1640 will be added to the top wells. The chamber will be incubated at 37°C with 5% CO_2 for 2 hours. The membranes will then be removed, and the cells present on the bottom well will be quantified by flow cytometry. For cell quantification, a fixed acquisition time of 30 seconds will be used per sample, and beads will be run during each experiment to ensure a reproducible acquisition. Test agents that induce a chemokinetic response in the lymphocytes, such a chemotactic response, preferably at least as effectively as R(-) etodolac, will be advanced to the next screen.

D. Induction of apoptosis in cancer cells

The pro-apoptotic activity of the test agents, e.g., the R-etodolac analogs, will be tested in primary CLL cells, as well as in other tumor cells, by using the MTT assay and by measuring the catalytic activation of caspase-3 using a fluorometric assay. In brief, cells will be incubated for up to 3 days in presence of serial dilutions of the selected test agents. Cells viability will be quantified in 96-well plates by adding the MTT reagent (at 1 mg/ml final) for 2-4 hours followed by SDS cell lysis and spectrophotometric analysis at 570 nm. Caspase catalytic activity will be measured in a 96-well plate assay using a specific fluorometric substrate (DEVD-AMC), after lysing the treated cells with a CHAPS/NP-40 lysing buffer followed by fluorometric analysis. Test agents that exhibit pro-apoptotic activity, e.g., that increase caspase activity, preferably at least as effectively as R(-)-etodolac, will be advanced to the next screen.

E. Lymphocyte depletion in mouse

The selected test agent will be orally delivered to mice of various backgrounds in a single dose of 25 and 100 mg/kg. The number of white blood cells will be counted using a neubauer chamber after 4, 24 hrs, 7 and 14 days post treatment. Test agents that do not lower white cell levels substantially, preferably no more than does R(-) etodolac, will be advanced to the next screen.

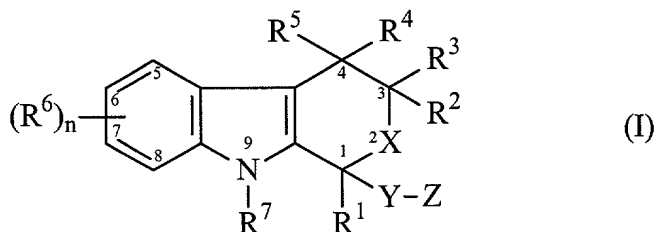
F. Tumor animal model

The anti-cancer and preventive activity of the R-etodolac analogs will be tested using the pristane-induced mouse myeloma model, and the transgenic adenocarcinoma mouse prostate (TRAMP) model. The mice will receive a diet supplemented with 0.05% to 0.5% of the selected test agent or control. The experimental diets will be in the form of sterile pellets containing the test agent (provided by Dyets Inc., PA). For prevention of cancer experiments in the mouse myeloma model, the diet will be initiated at the same time as the first pristane injection. For the transgenic prostate cancer model, the diet will begin at birth. For therapeutic experiments, the diet will begin in the TRAMP mice at week 10, when the first histological pathologic markers are usually observed. Analogs will advance to clinical trials or further development based on their activity to inhibit cancer in at least one of these screens.

All of the publications and patent documents cited hereinabove are incorporated by reference herein. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

What is claimed is:

1. A compound of formula (I):

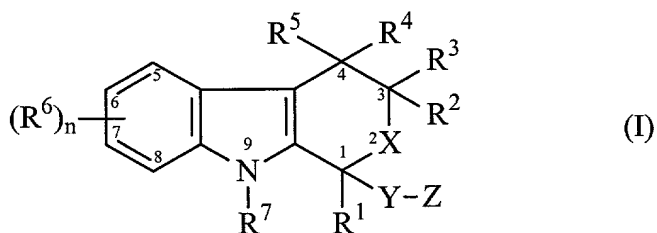


wherein R^1 is lower alkyl, lower alkenyl, (hydroxy)lower alkyl, lower alkynyl, phenyl, benzyl or 2-thienyl, R^2 , R^3 , R^4 and R^5 are the same or different and are each hydrogen or lower alkyl; each R^6 is individually hydrogen, lower alkyl, hydroxy, (hydroxy)lower alkyl, lower alkoxy, benzyloxy, lower alkanoyloxy, nitro or halo, n is 1-3, R^7 is hydrogen, lower alkyl or lower alkenyl, X is oxy and thio, Y is carbonyl, $(CH_2)_{1-3}$, $(CH_2)_{1-3}SO_2$ or $(CH_2)_{1-3}C(O)$, and Z is $(\omega-(4\text{-pyridyl})(C_2-C_4\text{ alkoxy}))$, $(\omega-((R^8)(R^9)\text{ amino})(C_2-C_4\text{ alkoxy}))$, wherein R^8 and R^9 are each H, $(C_1-C_3)\text{alkyl}$ or together with N are a 5- or 6-membered heterocyclic ring comprising 1-3 N(R^8), S or nonperoxide O; an amino acid ester of $(\omega-(HO)(C_2-C_4)\text{alkoxy})$, $N(R^8)CH(R^8)CO_2H$, 1'-D-glucuronyloxy; or $Y-Z$ is $(CH_2)_{1-3}R^8$; wherein R^8 is OH, $(C_2-C_4)\text{acyloxy}$, SO_3H , PO_4H_2 , $N(NO)(OH)$, SO_2NH_2 , $PO(OH)(NH_2)$, or tetrazolyl; or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1 wherein Z is the L-valine or L-glycine ester of 2-hydroxyethoxy.
3. The compound of claim 1 wherein Z is N-morpholinoethoxy.
4. The compound of claim 1 wherein each R^8 is H, CH_3 or i-Pr.
5. The compound of claim 1 wherein Z is $OCH_2CH_2N(CH_3)_3^+$.

6. A composition comprising the compound of claim 1 in combination with a pharmaceutically acceptable carrier.
7. The composition of claim 6 which is a tablet, granule or capsule.
8. The composition of claim 6 wherein the carrier is an aqueous vehicle.
9. The composition of claim 8 which is an aqueous solution.
10. A method of inhibiting cancer comprising administering an effective amount of the compound of claim 1 to a mammal afflicted with cancer.
11. A method of inhibiting cancer comprising administering an effective amount of the composition of claim 6 to a mammal afflicted with cancer.
12. The method of claim 10 or 11 wherein the cancer is prostate cancer.
13. The method of claim 10 or 11 wherein the cancer is multiple myeloma.
14. The method of claim 10 or 11 wherein the cancer is chronic lymphocytic leukemia.
15. The method of claim 11 wherein the composition is administered orally.
16. The method of claim 15 wherein an enterically coated dosage form is administered.
17. The method of claim 11 wherein the composition is administered parenterally.

18. The method of claim 11 wherein the composition is administered in combination with a chemotherapeutic agent.
19. The method of claim 12 wherein the composition is administered in combination with a chemotherapeutic agent.
20. The method of claim 18 wherein the chemotherapeutic agent is mitoxanthrone, prednisone, estramustine, vinblastine or a combination thereof.
21. The method of claim 12 wherein the chemotherapeutic agent is an anti-androgen.
22. The method of claim 21 wherein the anti-androgen is bicafutamide, nilutamide, flutamide, cycloproterone acetate or a combination thereof.
23. The method of claim 21 wherein the anti-androgen is leuprolide acetate, goserelin acetate or a combination thereof.
24. A therapeutic method comprising:
 - (a) evaluating the level of at least one of PPAR- γ , Mcl-1 or Bag-1 in cancer cells isolated from a patient afflicted with prostate cancer to determine if said level is sufficiently high so that said cells would be susceptible to inhibition by a compound of formula (I):



wherein R^1 is lower alkyl, lower alkenyl, (hydroxy)lower alkyl, lower alkynyl, phenyl, benzyl or 2-thienyl, R^2 , R^3 , R^4 and R^5 are the same or different and are each hydrogen or lower alkyl; each R^6 is individually hydrogen, lower alkyl, hydroxy, (hydroxy)lower alkyl, lower alkoxy, benzyloxy, lower alkanoyloxy, nitro or halo, n is 1-3, R^7 is hydrogen, lower alkyl or lower alkenyl, X is oxy and thio, Y is carbonyl, $(CH_2)_{1-3}$, $(CH_2)_{1-3}SO_2$ or $(CH_2)_{1-3}C(O)$, and Z is (ω -(4-pyridyl)(C_2 - C_4 alkoxy), (ω -((R^8)(R^9) amino)(C_2 - C_4 alkoxy), wherein R^8 and R^9 are each H, (C_1 - C_3)alkyl or together with N are a 5- or 6-membered heterocyclic ring comprising 1-3 N(R^8), S or nonperoxide O; an amino acid ester of (ω -(HO)(C_2 - C_4))alkoxy, N(R^8)CH(R^8)CO₂H, 1'-D-glucuronyloxy; or Y-Z is $(CH_2)_{1-3}R^8$ wherein R^8 is OH, (C_2 - C_4)acyloxy, SO₃H, PO₄H₂, N(NO)(OH), SO₂NH₂, P(O)(OH)(NH₂) or tetrazolyl; or a pharmaceutically acceptable salt thereof; and

- (b) administering to said patient an amount of a compound of formula (I) effective to inhibit said cells or to sensitize said cells to inhibition by administration of a chemotherapeutic agent.

25. The method of claim 24 wherein Y-Z is a pyridylalkyl ester, a morpholinoalkyl ester, an aminoalkyl ester or a hydroxyalkyl ester.

26. The method of claim 24 wherein Y-Z is a glucamine ester or N-(C_1 - C_4)alkyl-glucamine ester of CH₂CO₂H.

27. The method of claim 24 wherein Y-Z is the 1'-D-glucuronate ester of CH₂CO₂H.

28. The method of claim 24 wherein Y-Z is a water-soluble amide of CH₂CO₂H.

29. The method of claim 28 wherein Y-Z is an amino acid amide of $\text{CH}_2\text{CO}_2\text{H}$.
30. The method of claim 24 wherein the level of PPAR- γ is evaluated.
31. A method for determining the ability of a test agent to inhibit prostate cancer cells comprising contacting a population of cells from a prostate cancer cell line that expresses PPAR- γ with said agent and determining whether the agent increases the expression of PPAR- γ in said cells.
32. A method for determining the ability of a test agent to inhibit prostate cancer cells comprising contacting a population of cells from a prostate cancer cell line that expresses Mcl-1 or Bag-1 with said agent and determining whether the agent decreases the expression of Mcl-1 in said cells.
33. A method for determining the ability of a test agent to inhibit cancer comprising determining whether or not the agent competitively inhibits the receptor-mediated binding of radiolabeled etodolac to cancer cells.
34. The method of claim 33 wherein the cancer cells are etodolac sensitive.
35. The method of claim 33 wherein the etodolac is R(-)-etodolac.
36. The method of claim 31, 32, 33, 34 or 35 further comprising determining whether or not the agent increases the uptake of calcium by cancer cells.
37. The method of claim 36 further comprising determining whether or not the test agent can induce a chemokinetic response in a population of lymphocytes.
38. The method of claim 37 wherein the response enhances the ability of the lymphocytes to exhibit chemotaxis.

39. The method of claim 38 wherein the lymphocytes comprise B-CLL lymphocytes.
40. The method of claim 37 further comprising determining whether or not the test agent can induce apoptosis in cancer cells.
41. The method of claim 40 wherein the cancer cells are CLL cells.
42. The method of claim 40 comprising determining whether or not the test agent can increase caspase-3 activity.
43. The method of claim 40 further comprising determining whether or not the test agent lowers the white cell count of a test animal.
44. The method of claim 43 wherein the test animal is a mouse.
45. The method of claim 43 further comprising determining whether or not the test agent can inhibit cancer induction in the pristane-induced murine MLL model.
46. The method of claim 43 further comprising determining whether or not the test compound can inhibit cancer in the transgenic adenocarcinoma mouse prostate cancer model.
47. A method for determining the susceptibility of prostate cancer to treatment by a compound that activates PPAR- γ expression in prostate cancer cells comprising:
- (a) isolating prostate cancer cells from a human subject; and
 - (b) evaluating whether or not said cells express PPAR- γ at a level sufficient to render them subject to inhibition by said compound.

48. A method for determining the susceptibility of prostate cancer to treatment by a compound thereof that downregulates Mcl-1 or Bag-1 expression in prostate cancer cells comprising:

- (a) isolating prostate cancer cells from a human subject;
- (b) evaluating whether or not said cells express Mcl-1 at a level sufficient to render them subject to inhibition by said compound.

Abstract

The present invention provides novel indole derivatives useful to inhibit cancer or sensitize cancer cells to chemotherapeutic agents, radiation or other anti-cancer treatments.

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Figure 2

Sensitivity of CLL cells to Etodolac

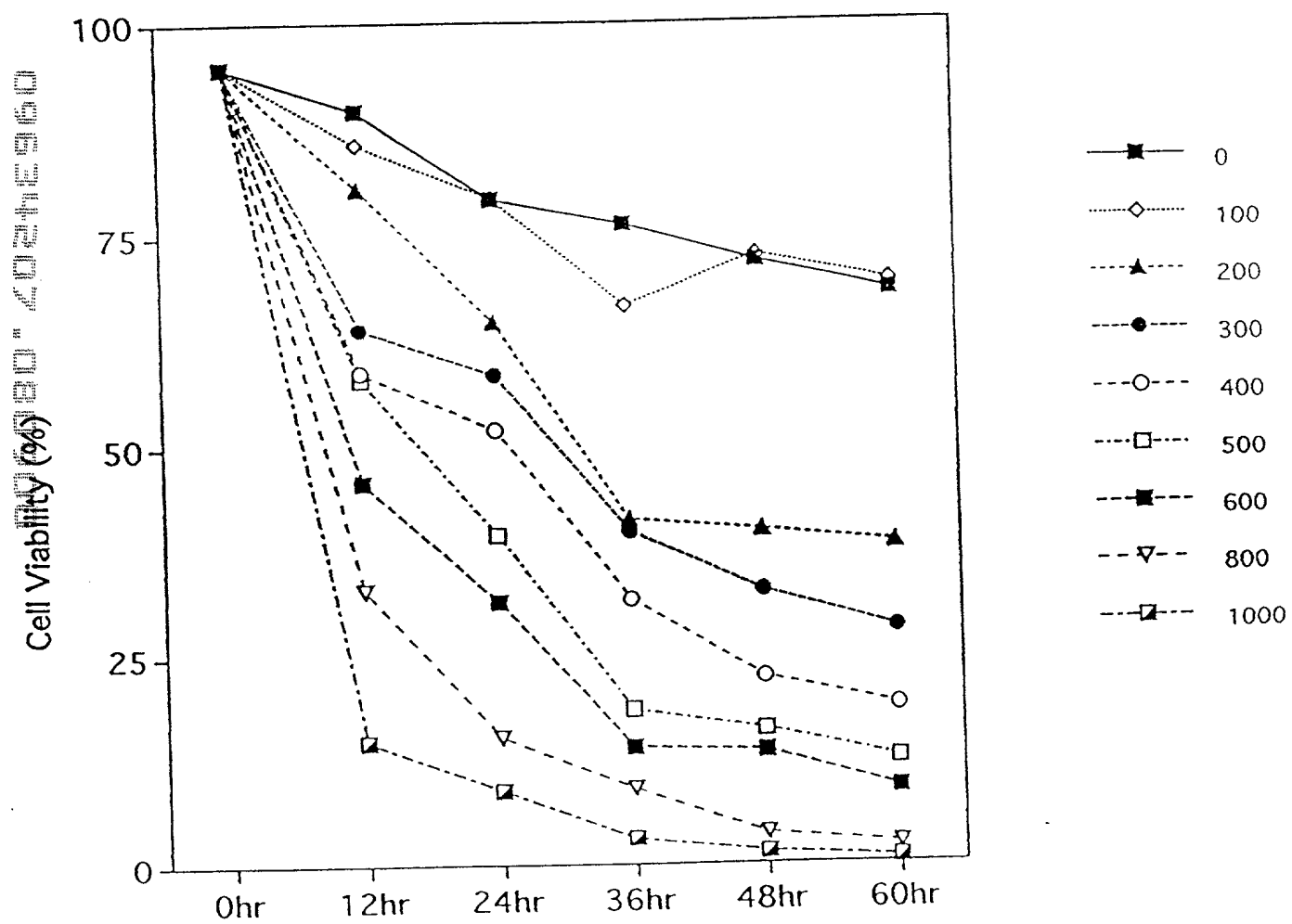


Figure 3

Etodolac (10 μ M) synergy with Fludarabine

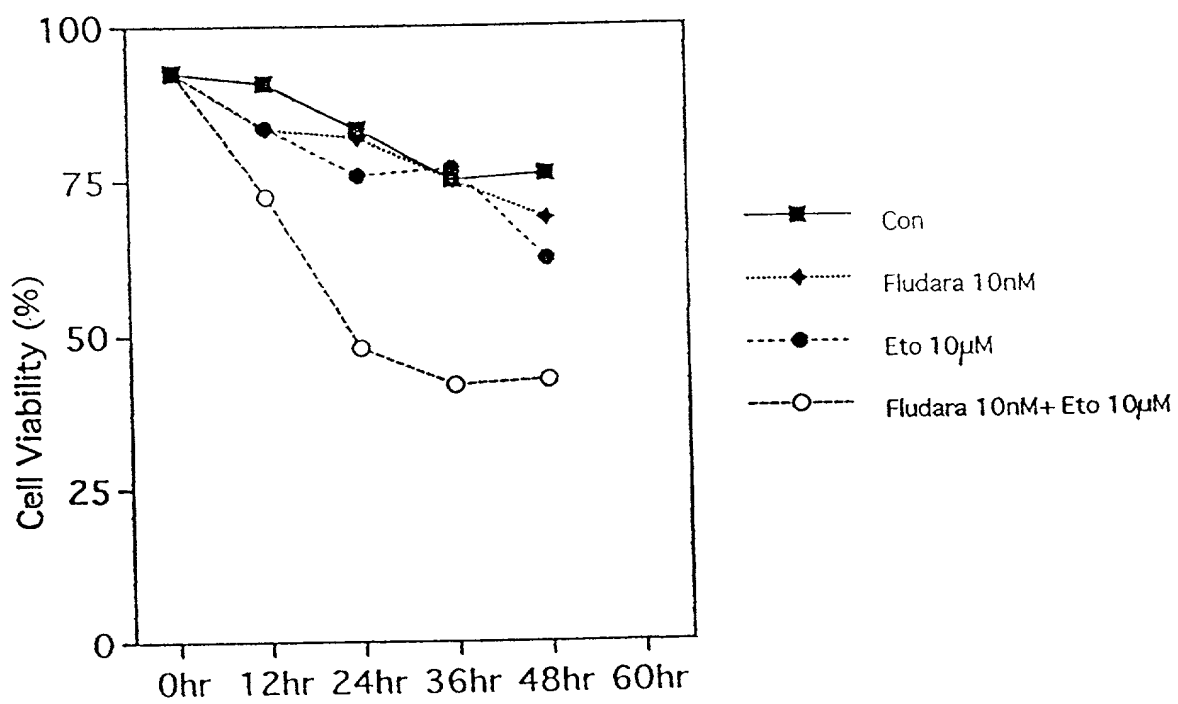


Figure 4

Etodolac (50 μ M) synergy with 2CdA and Fludarabine

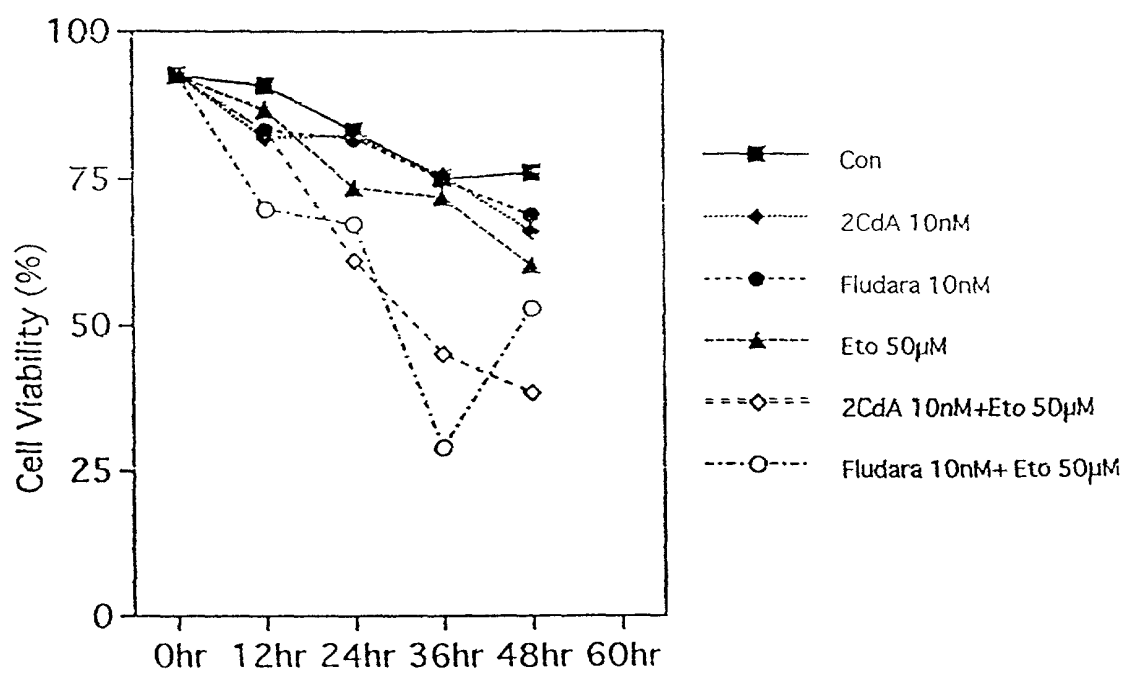


Figure 5

Sensitivity of CLL cells to S-Etodolac and R-Etodolac

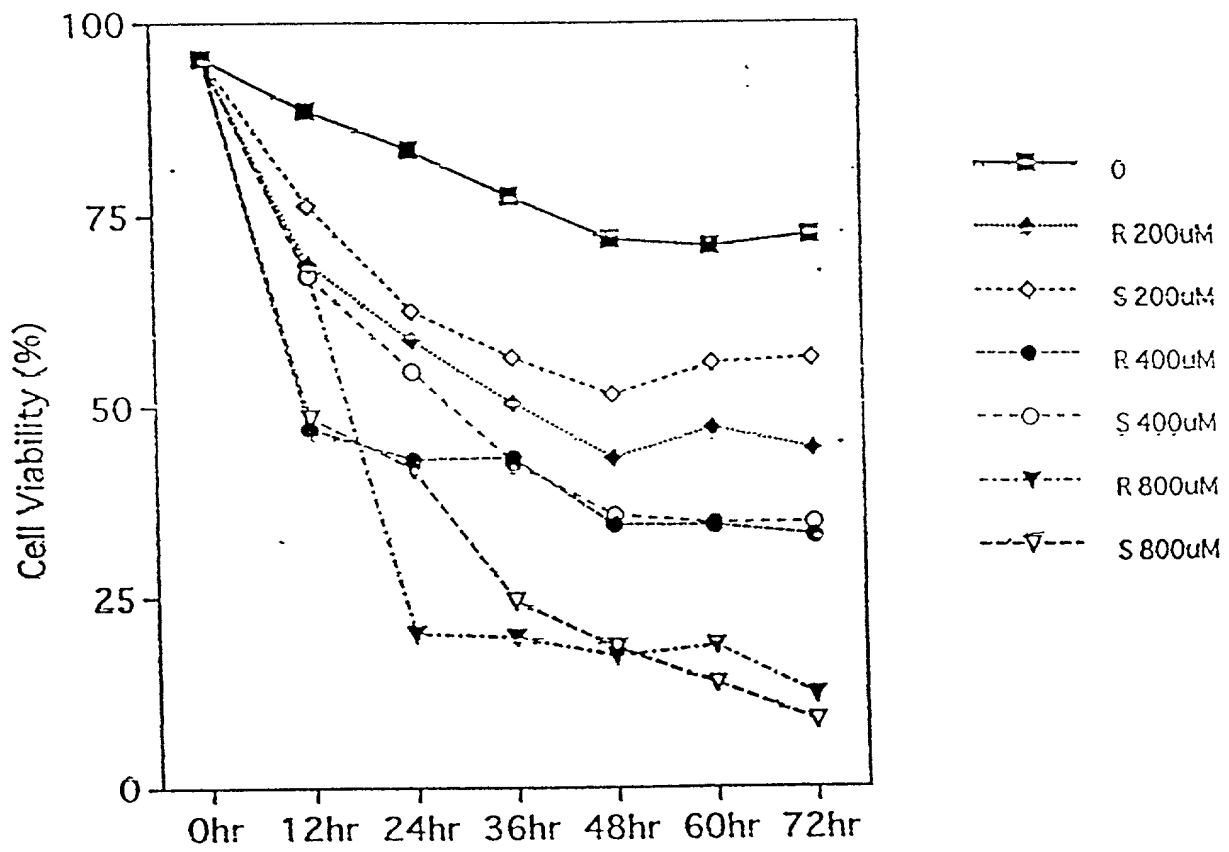


Figure 6

Cell Viability of CLL cells (J.K.) before and after Etodolac treatment

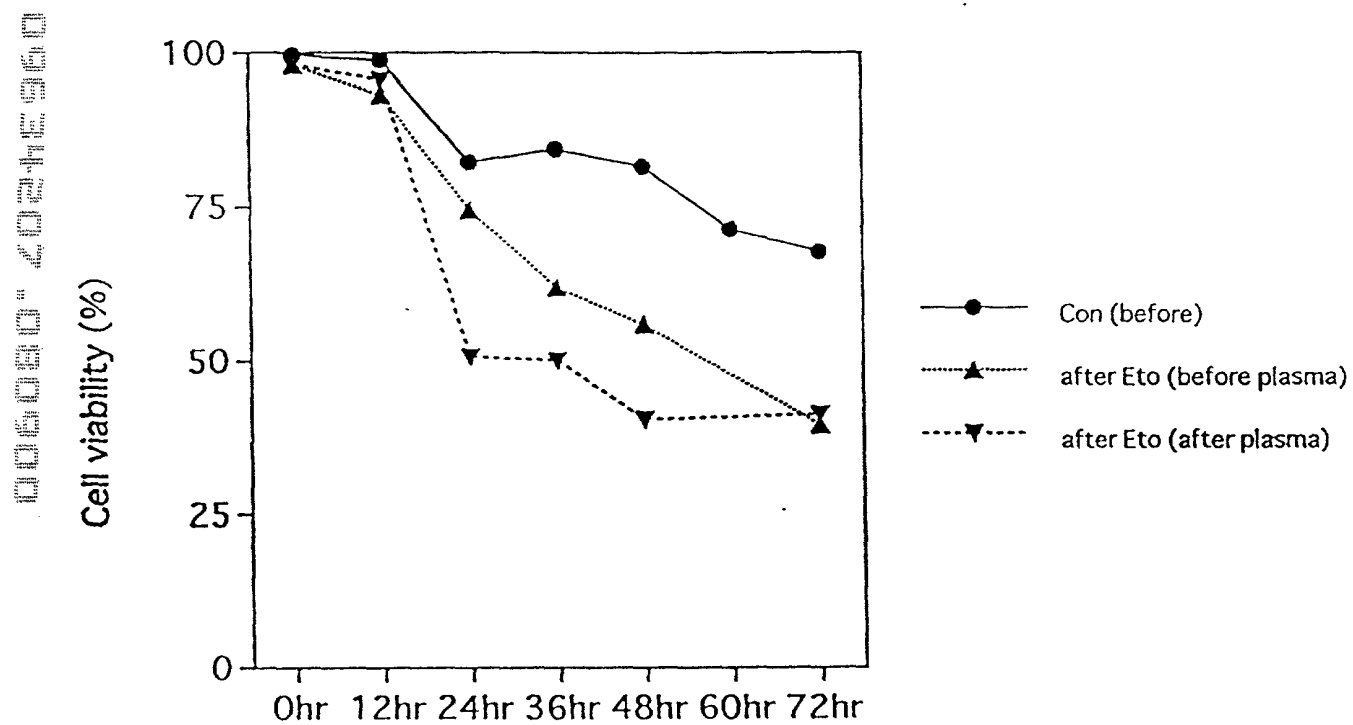


Figure 7

Cell Viability of CLL cells (N.A.) before and after Etodolac treatment

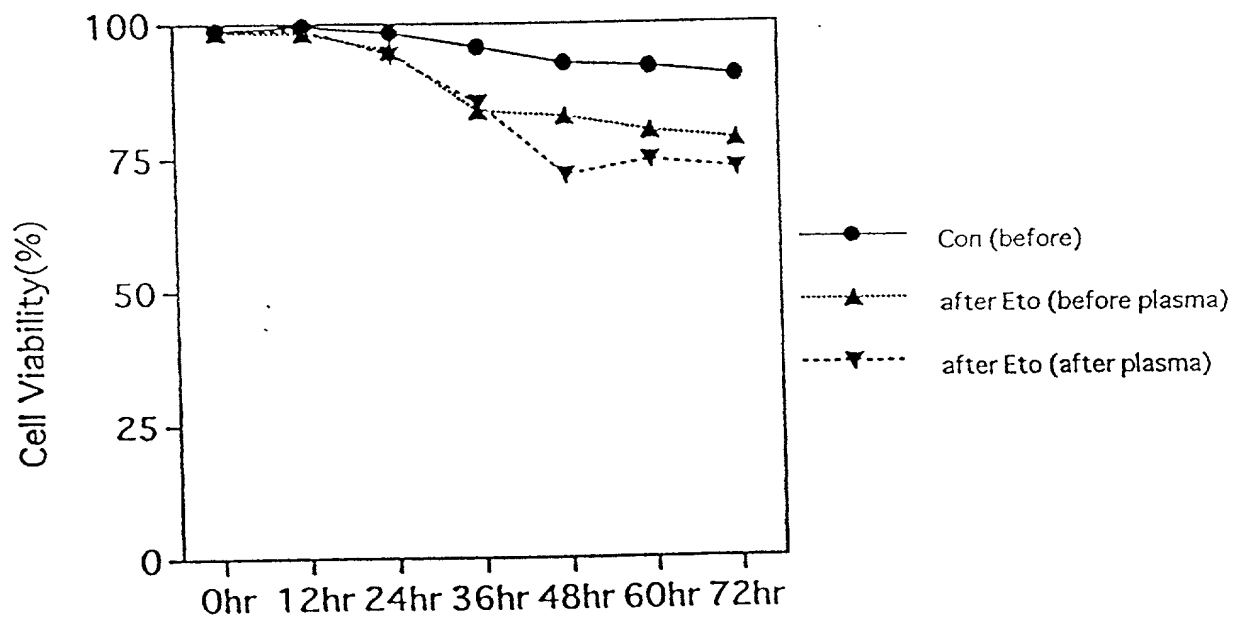
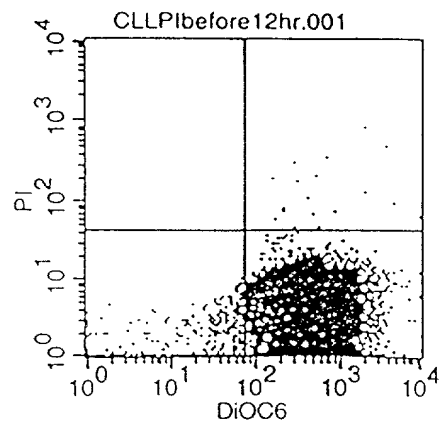
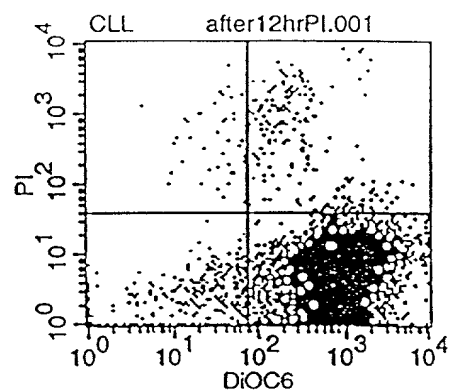


Figure 8

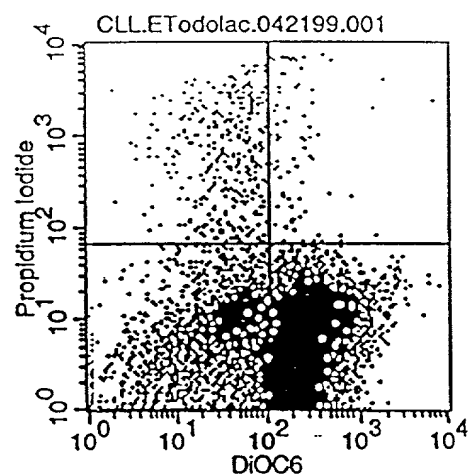
12hr
before



12hr
after



24hr
before



24hr
after

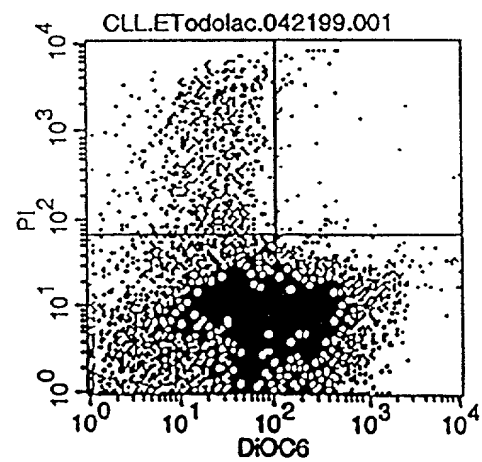


Fig 9

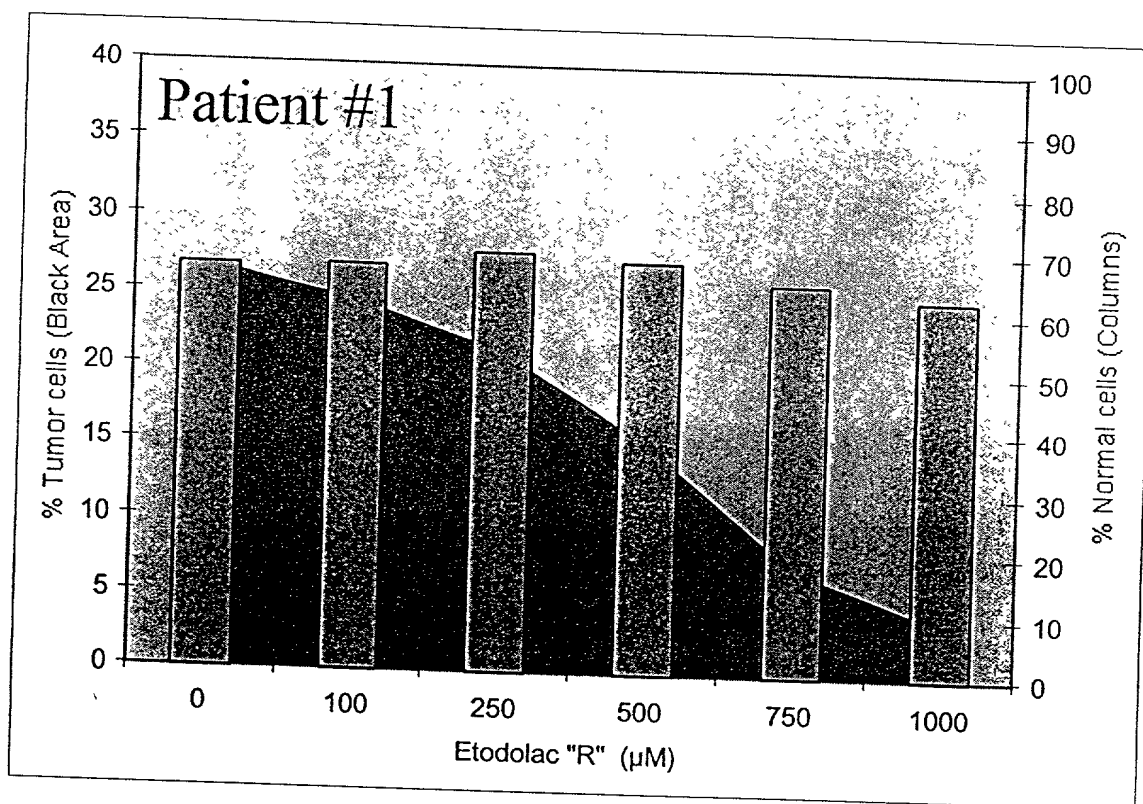


Fig. 10

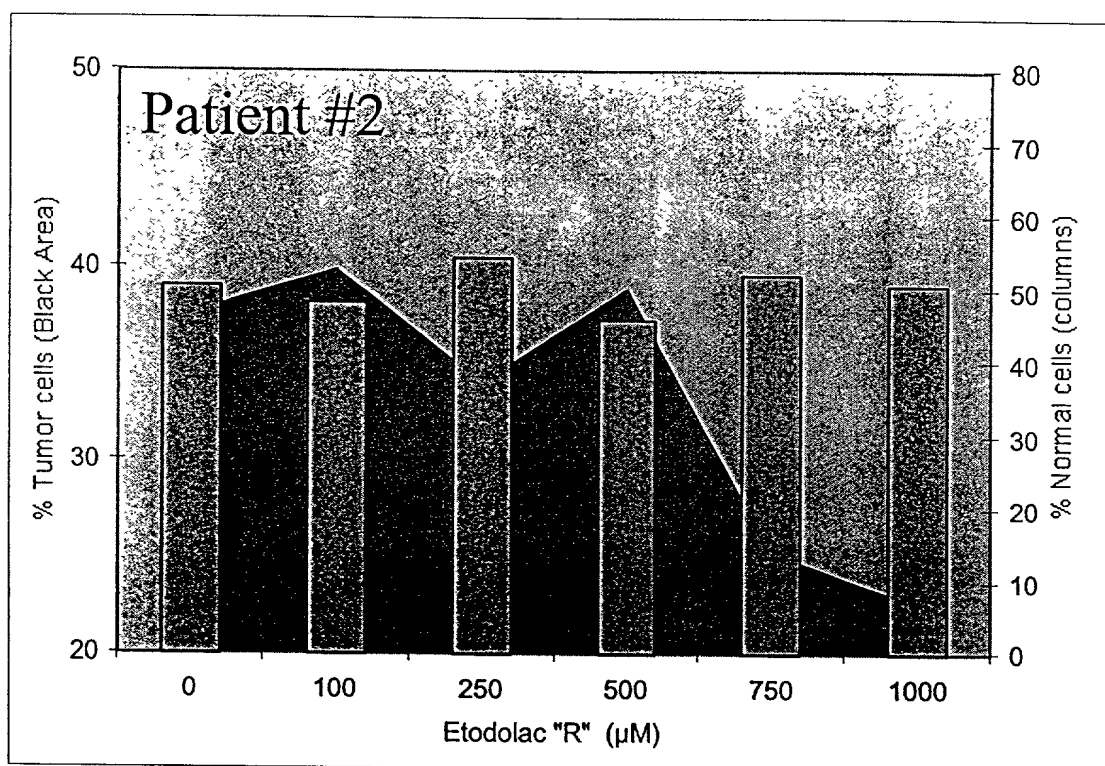


Figure 11

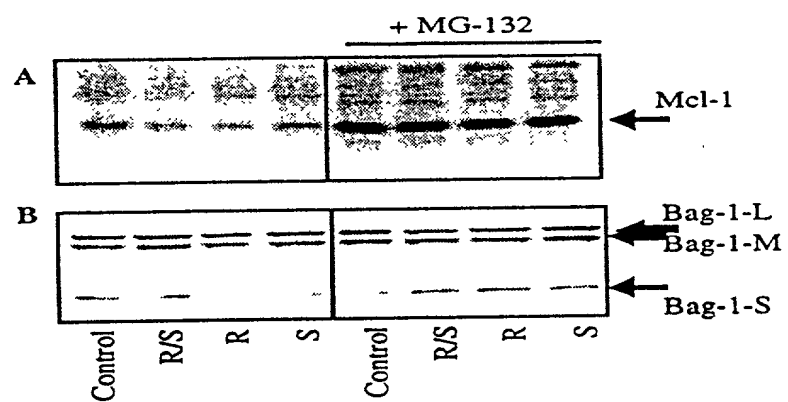


Figure 12

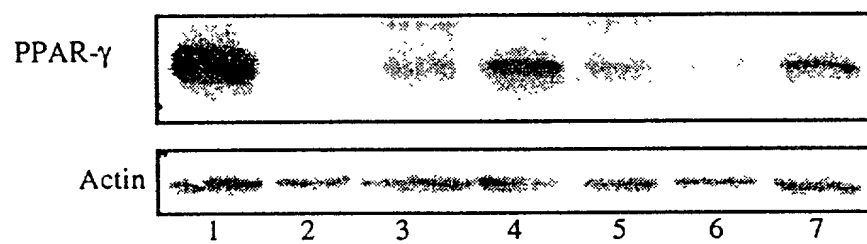


Fig. 13

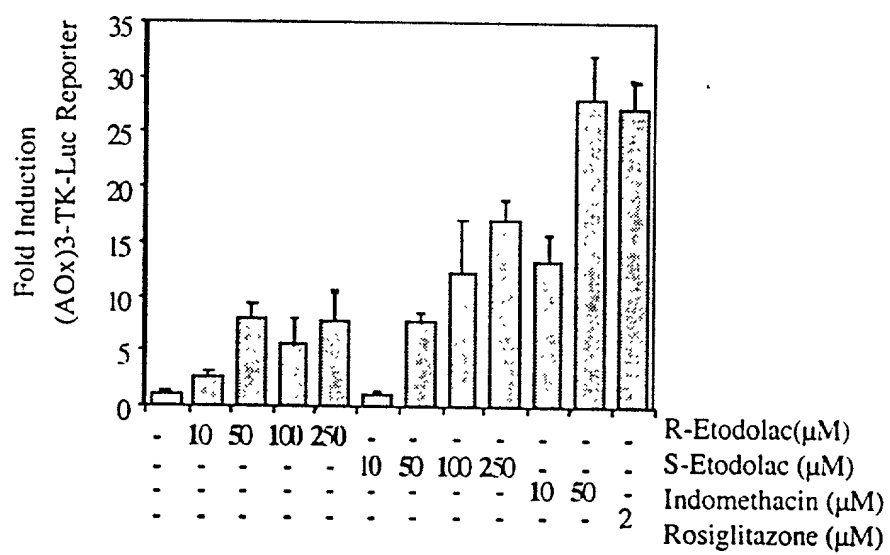


Figure 14

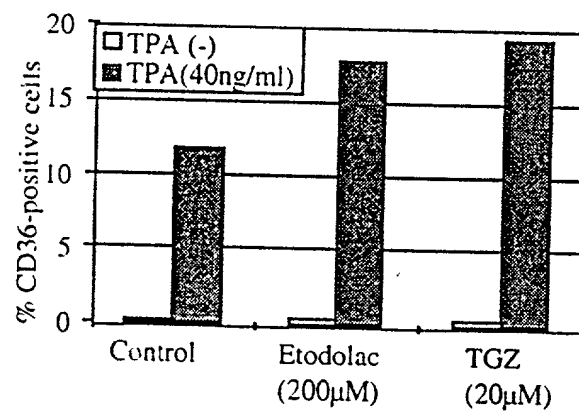
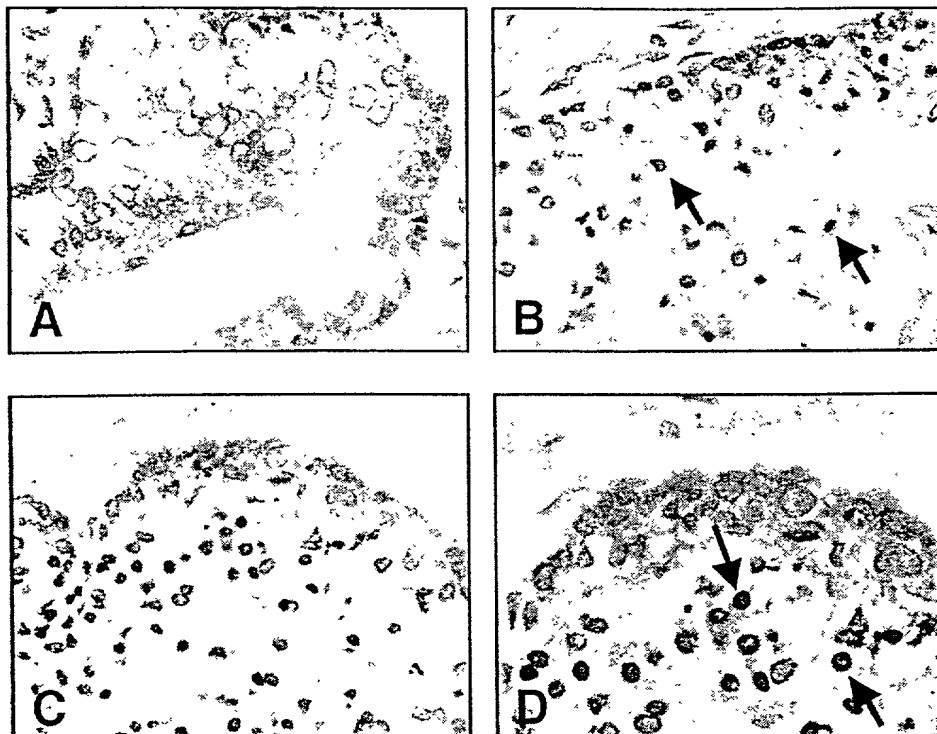


Figure 15



SCHWEGMAN ■ LUNDBERG ■ WOESSNER ■ KLUTH

United States Patent Application

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that

I verily believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: **INDOLE COMPOUNDS USEFUL FOR THE TREATMENT OF CANCER.**

The specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with 37 C.F.R. § 1.56 (attached hereto). I also acknowledge my duty to disclose all information known to be material to patentability which became available between a filing date of a prior application and the national or PCT international filing date in the event this is a Continuation-In-Part application in accordance with 37 C.F.R. § 1.63(e).

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on the basis of which priority is claimed:

<u>Application Number</u>	<u>Filing Date</u>	<u>Status</u>
09/360,020	July 23, 1999	Pending

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

<u>Application Number</u>	<u>Filing Date</u>
60/189,976	March 16, 2000

I hereby claim the benefit under 35 U.S.C. § 120 or 365(c) of any United States and PCT international application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. § 1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

No such claim for priority is being made at this time.

I hereby appoint the following attorney(s) and/or patent agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith:

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I hereby authorize them to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/organization/who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Schwegman, Lundberg, Woessner & Kluth, P.A. to the contrary.

Please direct all correspondence in this case to **Schwegman, Lundberg, Woessner & Kluth, P.A.** at the address indicated below:
P.O. Box 2938, Minneapolis, MN 55402
Telephone No. (612)373-6900

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of joint inventor number 1 : **Dennis A. Carson**
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Del Mar, CA 92014

Residence: **Del Mar, CA**

Signature: _____
Dennis A. Carson

Date: _____

Full Name of joint inventor number 2 : **Lorenzo M. Leoni**
Citizenship: **Switzerland**
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San Diego, CA 92122

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Signature: _____
Lorenzo M. Leoni

Date: _____

☒ Additional inventors are being named on separately numbered sheets, attached hereto.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Signature: _____ Date: _____
Howard B. Cottam

Full Name of inventor:
Citizenship:
Post Office Address:

Residence:

Signature: _____ Date: _____

Full Name of inventor:
Citizenship:
Post Office Address:

Residence:

Signature: _____ Date: _____

Full Name of inventor:
Citizenship:
Post Office Address:

Residence:

Signature: _____ Date: _____

§ 1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) prior art cited in search reports of a foreign patent office in a counterpart application, and
- (2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

- (1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or
- (2) It refutes, or is inconsistent with, a position the applicant takes in:
 - (i) Opposing an argument of unpatentability relied on by the Office, or
 - (ii) Asserting an argument of patentability.

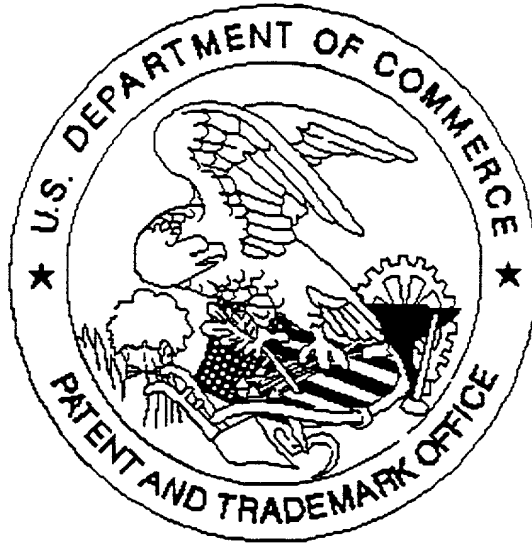
A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

- (1) Each inventor named in the application;
- (2) Each attorney or agent who prepares or prosecutes the application; and
- (3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

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